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Patterns of cytokine gene expression of naïve and memory T lymphocytes in vivo

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Abstract

Large-scale lymphocyte recirculation occurs only at the level of secondary lymphoid tissue. Cells enter lymph nodes via afferent lymph from the tissue and via arterioles from the blood. They exit only via the efferent duct. Afferent and efferent lymphocytes have distinct phenotypes; afferent lymphocytes have a 'memory' phenotype, being $CD62L^{-}/CD45RA^{-}$ and expressing high levels of CD2 and CD11a; efferent cells are largely 'naïve', being $CD62L^{+}/CD45RA^{+}$ with low levels of CD2 and CD11a. We will show that functionally the efferent lymphocytes, like cells from the blood and spleen, can be activated in vitro only by dendritic cells. However, afferent lymphocytes are less stringent in their activation requirements and can be stimulated by both macrophages and dendritic cells. To explain these functional differences we have developed a multiprobe RNAase protection assay for 13 sheep cytokines (IL-1β, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, GMCSF, IFNγ, TGFβ and TNFα) and two housekeeping genes (ATPase and GADPH). We have used this assay to measure the constitutive expression of cytokine mRNA in MACS-purified CD4+ and CD8+ T lymphocytes from both lymphoid compartments.

Keywords: Cytokines; T lymphocytes; Memory figure

1. Introduction

The physiological processes of the immune system, especially adaptive immune responses, are dynamic processes dependent upon the co-ordinated interaction of antigen-trapping accessory cells and lymphocytes in specialised peripheral lymphoid organs such as lymph nodes. The patterns of lymphocyte recirculation through different immunological compartments have been well defined in the sheep. Blast cells from peripheral nodes home to the spleen and lymph nodes, and within this small recirculating lymphocyte pool there are subsets which preferentially recirculate thorough the skin, peripheral nodes or mucosal associated lymphoid tissue (Mackay et al., 1988; Abernethy et al., 1991). The afferent lymph selectively conveys T lymphocytes, a small proportion of B lymphocytes, afferent lymph dendritic cells (ALDCs) and macrophages (Hall and Morris, 1965; Smith et al., 1970). The ALDCs are professional APCs involved in the carriage of antigen in an immunogenic form from the skin to the draining lymph node.

Efferent lymph contains only lymphocytes that enter the lymph node, either from the blood or the afferent lymphatics of the node and leave via the efferent, lymphatics within the lymph plasma, ultimately back into the blood (Hall and Morris, 1962; Smith et al., 1970). The lymph node is a central focus of the immune

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system and the use of cannulation of the afferent and efferent lymphatics provides a unique approach to the in vivo analysis of immune cell populations within the different immunological microenvironments of single lymph nodes in the sheep. There are considerable phenotypic and functional differences between the cells in blood and afferent lymph suggesting that there is selectivity in the extravasation of cells from the blood across the endothelium into the peripheral tissues. Emigration of afferent and efferent T lymphocytes, with their distinct phenotypes and function, can be demonstrated by the circulation of naïve and memory T lymphocytes within an individual animal (Mackay et al., 1996).

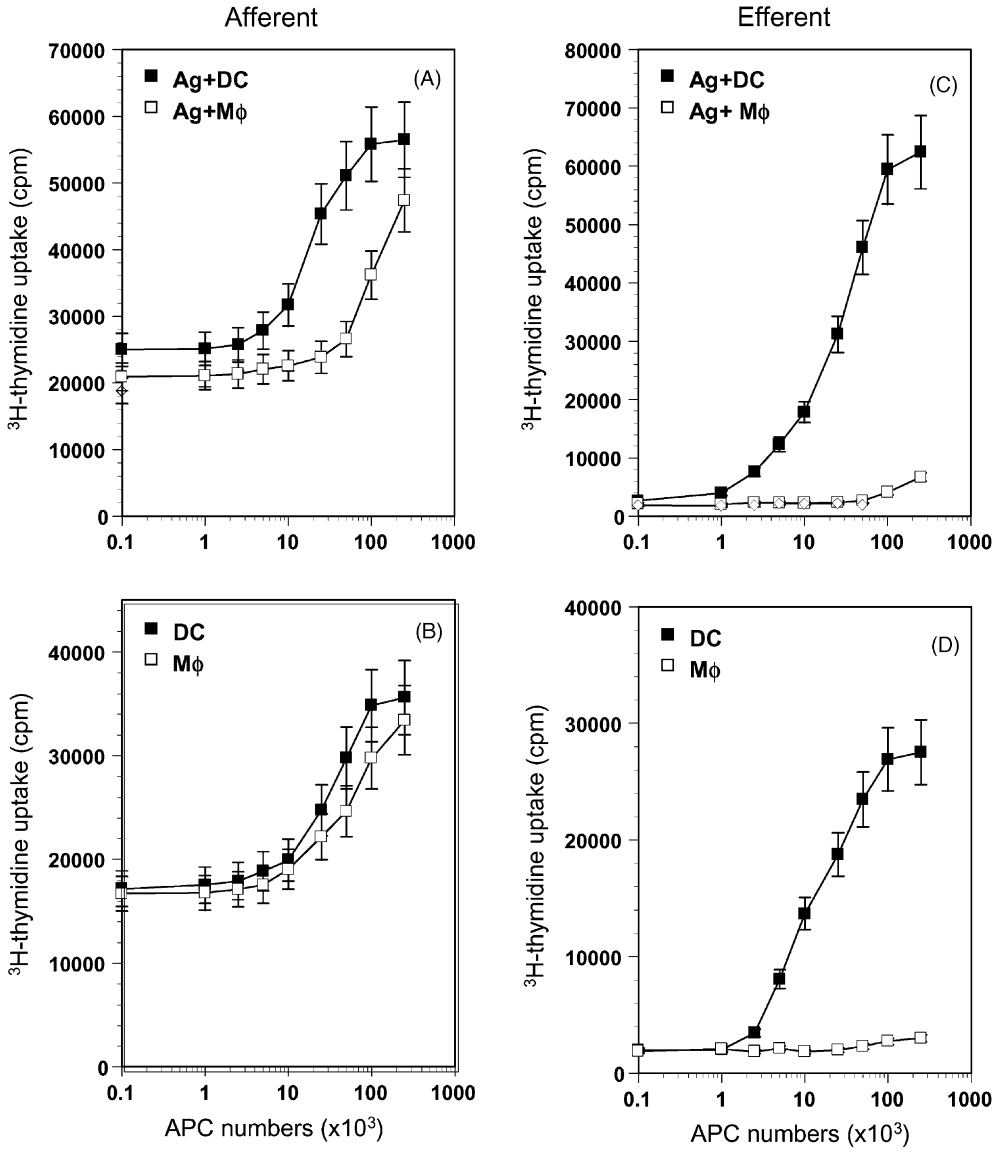

Fig. 1. Proliferation of CD4+ lymphocytes from both afferent and efferent lymph in vitro. In vitro proliferation assays of CD4+ T cells from afferent (A and B) or efferent (C and D) lymph stimulated with 25 μg/ml ovalbumin (A and C) or allogeneic APC (B and D). 5×10^4 lymphocytes were cultured with irradiated afferent DC (DC) or mammary macrophages (Mϕ) for 5 days and then labelled with ^{3}H-thymidine for 5 h.

The blood contains T lymphocytes of both naïve and memory phenotypes though afferent lymph T lymphocytes are mainly of the memory cell phenotype and efferent T lymphocytes draining the lymph node are mainly of a naïve phenotype. Mature lymphocytes, both CD4+ and CD8+, recirculate between the blood and the tissues via the lymph nodes. Immunological memory is disseminated from the node via the efferent lymph, where exit of T lymphocytes following antigen stimulation is non-random despite the fact that cells from the efferent lymph have a 'naïve' phenotype.

A total of 90% of the lymphocytes within a lymph node are derived from the blood, the remainder are from lymphocyte proliferation within the node and afferent lymph (Hall and Morris, 1965). CD4+ cells are selectively enriched over CD8+ T lymphocytes in lymph nodes.

To investigate the relationships between functional activities of lymphocytes and their migratory properties, we have developed a multiprobe RNAase protection assay (RPA) for 13 sheep cytokines (IL-1β, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, GMCSF, IFNγ, TGFβ and TNFα), as well as two housekeeping genes (ATPase and GADPH). Cannulation of the prefemoral ''pseudo-afferent'' and efferent lymphatics of sheep allows the isolation of 'resting' trafficking lymphoid cells to be analysed for their constitutive cytokine mRNA expression.

2. Naïve, memory and effector lymphocytes

Afferent lymph contains ALDCs, T lymphocytes and B lymphocytes, all migrating from the tissues to the local lymph node. The afferent T lymphocytes express an activated phenotype ($CD2^{HI}$ $CD58^{HI}$ $CD44^{HI}$ $CD11a^{HI}$, high level expression of MHC II DQ^{+} and CD25) and high levels of adhesion molecules but are $CD45RA^{-}$ compared to efferent lymph cells (Mackay et al., 1990, 1992; Abitorabi et al., 1996). In terms of function they respond to soluble antigen (Fig. 1A) and alloantigen presented by both ALDCs and macrophages (Fig. 1B).

Efferent lymph contains >99% lymphocytes with a high proportion of CD4+ T lymphocytes and B lymphocytes compared to CD8+ and γδ T lymphocytes. T lymphocytes isolated from efferent lymph ($CD2^{LO}$ $CD58^{LO}$ $CD44^{LO}$ $CD11a^{LO}$, class II MHC DQ^{-} and $CD25^{-}$) are $CD45RA^{+}$ and express low levels of adhesion molecules (Mackay et al., 1990, 1992; Abitorabi et al., 1996). These T lymphocytes are highly responsive to both soluble antigen and alloantigen when presented by ALDCs. They respond very weakly to soluble antigen presented by macrophages and are non-responsive to allogenic macrophages in a mixed leukocyte reaction (Fig. 1D).

Using MACS-purified CD4+ and CD8+ T lymphocytes from afferent and efferent lymph, the constitutive expression of cytokine mRNA in both cell populations using a multiprobe RPA was analysed. Preliminary results identified T lymphocytes from ''resting'' afferent lymph that expressed mRNA for the cytokines IL-1β, IL-3, IL-8, IFNγ, TNFα and TGFβ using this assay system (Fig. 2). With T lymphocytes

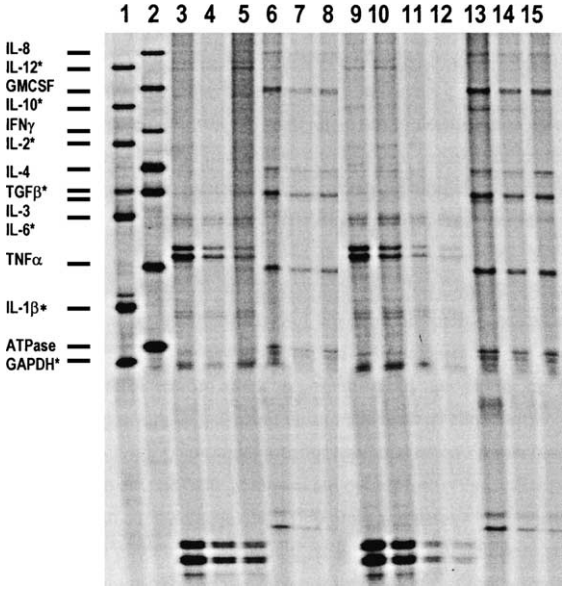

Fig. 2. Measurement of cytokine mRNA levels using an RNAase protection assay. The RNAase protection assay is a sensitive and specific method for the quantitation of mRNA species. The probe templates were generated by RNA polymerase directed synthesis of ^{32}P-labelled anti-sense RNA from a cDNA template. The purified-labelled probes were hybridised with the target RNA isolated from both afferent (lanes 9–15) and efferent (lanes 3–8) lymph cells. After hybridisation was completed, ribonucleases specific for ssRNA digested unhybridised RNA and probe. The ''RNAase-protected'' probes and undigested probe markers (lanes 1 and 2) were resolved on a denaturing PAGE and were visualised by phosphor imaging. Probe set 1* was used with the samples in lanes 3–5 and 9–12. Probe set 2 was used with the samples in lanes 6–8 and 13–15.

from ''resting'' efferent lymph expressed mRNA for the cytokines IL-2, IL-3, IFNγ, and TNFα were detected (Fig. 2).

3. Discussion

'Resting' efferent lymph T lymphocytes with their naïve phenotype require activation by ALDCs to respond to soluble antigen, yet 'resting' afferent lymph T lymphocytes do not require ALDCs for activation, as they are activated by macrophages, though they still also respond to ALDCs. This clearly demonstrates that afferent lymph responder cells have 'immunological memory', thus enabling them to respond to antigen presentation by macrophages. Conversely with 'resting' efferent lymph, there are few unprimed responder cells able to respond to soluble antigen without stimulation by ALDCs. Thus afferent lymph T lymphocytes demonstrate a lower activation threshold, i.e. 'memory', which is functionally illustrated by their ability to respond both to ALDCs and mammary macrophages. In contrast efferent lymph T lymphocytes have a higher activation threshold, confirmed by their ability to respond to ALDCs but not macrophages. Both sets of lymphocytes act in response to stimulation from ALDCs and yet have distinctly different phenotypes.

The responder cells of efferent lymph with their naïve phenotype have recirculated from the blood but have no 'immunological memory' and a higher activation threshold, which is only overcome by presentation of both soluble antigen and alloantigen by ALDCs and not macrophages. 'Naïve' T lymphocytes travel to areas of secondary lymphoid organs in search of antigen presented by dendritic cells. T lymphocyte memory is demonstrated by afferent lymph cells ability to proliferate in response to soluble antigen presentation by macrophages but the presence of memory effector cells is demonstrated by their ability to respond to alloantigen presentation by both ALDCs and macrophages though to a lesser extent than with soluble antigen.

Further work needs to be done to map and define naïve, memory and effector cells before the issue of naïve and memory phenotype and distribution can be resolved. These new RPA multiprobe sets provides valuable tools for the simultaneous quantitative determination of gene expression of multiple ovine cytokines of both constitutive and inducible type.

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